

Expression of the *Engrailed* Gene Reveals Nine Putative Segment-Anlagen in the Embryonic Pleon of the Freshwater Crayfish *Cherax destructor* (Crustacea, Malacostraca, Decapoda)

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Abstract. Segment formation in the embryonic pleon of the freshwater crayfish *Cherax destructor* was analyzed by using the monoclonal antibody mAb 4D9 against the product of the segment-polarity gene *engrailed*. As in other body regions, *engrailed* is expressed in transverse stripes in the posterior portion of segments in the pleon. Nine *engrailed* stripes are formed in the pleon. The anterior six stripes correspond to the six pleon segments of adult eumalacostracan crustaceans. The uropods are clearly the appendages of the sixth pleon segment. The seventh *engrailed* stripe marks the anlage of a seventh ganglion. Stripes eight and nine are transient and disappear before morphogenesis begins. The *engrailed* stripes seven to nine are interpreted as vestiges of ancestral segments. The seventh segment anlage is thus a recapitulation of the seventh pleonic segment, which is retained in recent adult leptostracans and is considered to be part of the malacostracan ground plan. The stripes eight and nine might point still further back into the phylogeny of crustaceans or even mandibulates. The use of rhodamine-labeled phalloidin reveals that the terminal ganglion of adult crayfish is the fusion product of the anlagen of the sixth and seventh pleonic ganglia and an eighth hemiganglion that is devoid of *engrailed* expression.

Introduction

The Malacostraca constitutes a monophyletic taxon well defined by several apomorphic characters such as a

characteristic tagmosis, the position of the gonopores, the subdivision of the stomach into specific functional units, and a ring of 19 embryonic ectoteloblasts (for review and discussion of different views see Dahl, 1992; Wägele, 1992). Within the Malacostraca, the Leptostraca possess a pleon (abdomen) consisting of seven segments and a telson. In contrast to all anterior segments, the seventh pleonic segment is limbless. The other malacostracan groups, unified as the Eumalacostraca (*sensu* Grobben, 1892), have only six pleomeres, all equipped with limbs. The posteriormost limbs are the uropods which, together with the flattened telson, form the tail fan. The general view is that the pleon of the Leptostraca represents the plesiomorphic condition, and the loss of a pleon segment and the evolution of the tail fan are considered to be derived characters of the Eumalacostraca (*e.g.*, Lauterbach, 1975; Hessler, 1983). However, there has been some controversy over which pleomere has been lost in the course of evolution and to which segment the uropods belong. On the basis of anatomical and paleontological data, Siewing (1956, 1963) argued that the uropods might be the appendages of the seventh pleomere and that the sixth (penultimate) pleomere has been lost in most eumalacostracans. Based on her embryological studies in mysidaceans, Manton (1928a, b), in contrast, suggested that the uropods belong to the sixth pleomere and that the original seventh is fused to the sixth pleomere. Although it was shown in the meantime that paleontological data do not support Siewing's suggestions (Dahl, 1983)—and although Manton's view has been adopted by many carcinologists such as Lauterbach (1975), Hessler (1983), and Dahl (1992)—the problem is still far from being settled

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(see, for instance, the discussion after the 1983 paper by Hessler) and a different approach seemed to be required to clarify this issue.

Within the arthropods, insects and crustaceans have the segment-polarity gene *engrailed* already expressed in transverse stripes at the posterior margin of embryonic segments before morphogenesis takes place (e.g., Patel *et al.*, 1989; Manzanares *et al.*, 1993; Scholtz *et al.*, 1993, 1994; Patel, 1994). Therefore, it is a suitable marker for the analysis of the terminal regions of arthropods where segmentation is obscured by morphological rearrangements and the loss of segmental structures in the adult. Several studies have used *engrailed* to analyze the segmentation of the head in insects and crustaceans (e.g., Fleig, 1994; Schmidt-Ott *et al.*, 1994; Scholtz, 1995), but the caudal segments have been analyzed only in insects (e.g., Kuhn *et al.*, 1992; Schmidt-Ott *et al.*, 1994).

In the present investigation, I used the anti-*engrailed* antibody mAb 4D9 (Patel *et al.*, 1989) to analyze the segmentation of the embryonic pleon of a eumalacostracan, the Australian freshwater crayfish *Cherax destructor*. I found that, posterior to the six pleomeres typical for adult eumalacostracans, three additional vestigial segment anlagen occur in front of the telson. Furthermore, a true seventh pleonic ganglion and an eighth partial ganglion are formed embryologically. These fuse with the sixth pleonic ganglion anlage to form the terminal ganglion of the adult animal. I interpret these findings as recapitulations of ancestral characters, and they shed new light on the evolutionary transformation of crustacean segmentation patterns.

Material and Methods

The rearing and maintenance of embryos of the Australian freshwater crayfish *Cherax destructor* were described by Sandeman and Sandeman (1991). Their paper also defines the embryonic stages in percent of development and the postembryonic stages (e.g., PO I) that are used in the present investigation. Immunocytochemistry and fluorescent staining were described in detail in Scholtz *et al.* (1994) and Scholtz (1995).

Results

A short summary of previous investigations

The cell lineage in the ectoderm of the germ band of *Cherax* was described in a previous study (Scholtz, 1992). As in most other malacostracans, the largest part of the germ band is formed by stem cells in the posterior growth zone, the ectoteloblasts. The ectoteloblasts produce transverse cell rows in an anterior direction by highly unequal divisions. Thirteen of these rows are formed (eI to eXIV) before the ectoteloblasts divide equally into the fourteenth

and fifteenth rows (eXIV and eXV). Each row (eI to eXIV) cleaves twice, forming four regularly arranged descendant rows. The intersegmental furrow, separating two adjacent segments, is formed within the descendants of one original ectoderm row. In contrast to all other rows, row fifteen cleaves rapidly several times, forming a field of cells in a grid-like arrangement.

In *Drosophila*, the segment-polarity gene *engrailed* plays a crucial role in specifying the fate of the cells in the posterior part (compartment) of segments and in establishing the segmental boundaries (Lawrence, 1992). The expression pattern of *engrailed* is very similar in different insect and crustacean species. Therefore, a conserved function of the *engrailed* gene throughout the arthropods has been suggested (Patel, 1994). The basic modes of pleonic *engrailed* stripe formation described in the following correspond to those reported for other body regions of *Cherax* (Scholtz *et al.*, 1993; Scholtz, 1995) and for other crustacean species (Patel *et al.*, 1989; Scholtz *et al.*, 1993, 1994). In the post-naupliar germ band of *Cherax* and other malacostracans, *engrailed* is expressed in the anterior descendants of each ectoderm row, and the intersegmental furrow is formed posterior to the *engrailed* stripes.

The formation of engrailed stripes

Nine *engrailed* stripes are formed in the embryonic pleon of *C. destructor* (Fig. 1). The first pleonic stripe appears in ectoderm row eIX and the sixth stripe in row eXIV. Stripes seven to nine are formed within the derivatives of row eXV.

The stripes appear in a strictly anteroposterior sequence (Fig. 1). At about 40% to 42% development the first *engrailed* stripe is formed and indicates the posterior margin of the prospective first pleomere (Fig. 1A). The ninth pleonic *engrailed* stripe appears at about 65% development (Fig. 1D, E). The formation of each individual stripe starts close to the midline and proceeds laterally (Fig. 1D). The initial distance between the last two *engrailed* stripes of any stage is one row of *engrailed* negative cells. This is also true for stripes seven to nine (Fig. 1E). Stripes one to seven are associated with the complex metameric repeated cleavage pattern in the post-naupliar germ bands of *Cherax*. The cell division pattern in the area of stripes eight and nine, although not analyzed in detail, is somewhat different (Scholtz, 1992).

Initially, all stripes are one cell wide (Fig. 1). Also, at least pleonic stripes one to eight (not confirmed for pleonic stripe nine) pass through a transient widening phase caused by divisions of the *engrailed*-positive cells (Fig. 1B, C). The widening phase is followed by narrowing to a one-cell width again due to the loss of *engrailed* expression in posterior cells in the stripe (Fig. 1B, C). After nar-

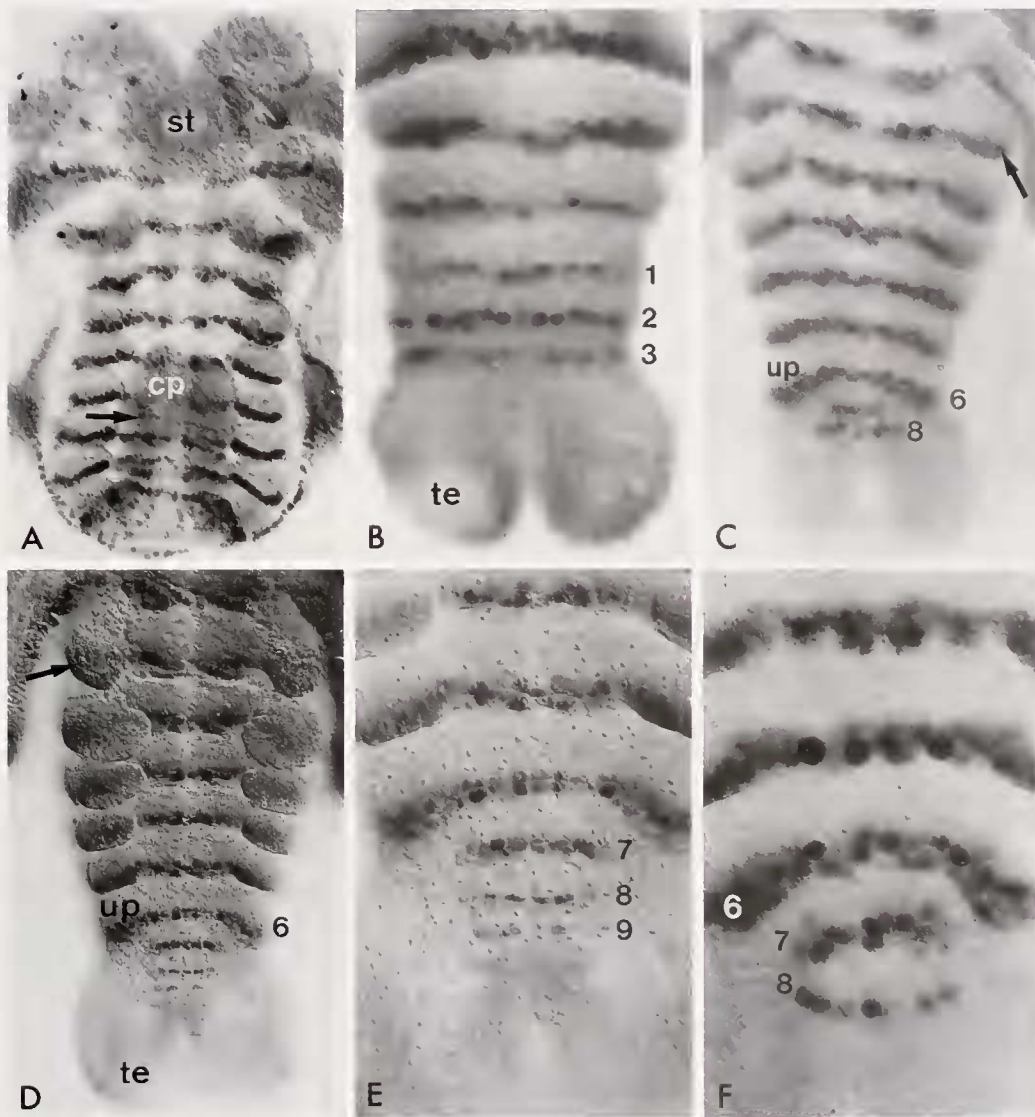


Figure 1. Formation of *engrailed* stripes: st, stomodeum; cp, caudal papilla; te, telson anlage; up, uropods. (A) Germ band at 40% to 42% development. All *engrailed* stripes of head and thorax are formed. The *engrailed* stripe marking the first pleonic segment appears (arrow) on the ventral side of the ventrally flexed caudal papilla. (B) Posterior part of the caudal papilla of an embryo at 45% development (ventral view). The *engrailed* stripes up to the third pleonic segment (1, 2, 3) are formed. The posteriormost stripe (3) is one cell wide. The next anterior stripe (2) is in the narrowing phase after the initial widening. The stripe of the first pleonic segment (1) is in the phase of secondary widening in correlation with the formation of the intersegmental furrow and the limb buds (for details see Scholtz *et al.*, 1993). (C) Posterior part of the caudal papilla of an embryo at about 60% development (ventral view); *engrailed* stripes seven and eight are formed. Stripe eight is in the first widening phase; stripe seven has already narrowed to one cell width. The uropods begin to appear in the area of the sixth *engrailed* stripe (sixth pleonic segment). Note the buds of the first pleopods (arrow), which are lacking in the adults of *Cherax* (comp. Fig. 1D). (D) Posterior part of the caudal papilla of an embryo at about 65% development (ventral view). The ninth *engrailed* stripe appears following a mediolateral sequence of *engrailed* expression. The ninth stripe is the posteriormost area of *engrailed* expression, the posterior margin of the telson anlage does not express *engrailed*. The arrow points to the buds of the first pleopods. (E) Closeup of the same preparation as in (D); 7 to 9 seventh to ninth pleonic *engrailed* stripes. (F) Posterior part of the caudal papilla of an embryo at 70% development (ventral view). Neuronal *engrailed* expression begins in the area of the seventh pleonic stripe (7). The cells of *engrailed* stripe eight begin to cease *engrailed* expression; stripe nine has disappeared.

rowing, stripes one to seven widen during the morphogenesis of segmental structures such as intersegmental furrows, ganglia, and limb buds (Fig. 1). Stripes eight and nine disappear before widening takes place (Fig. 1F).

Stripes one to six surround the caudal papilla and form complete circles of about 40 *engrailed*-positive cells (Fig. 1, 2A). Thus, *engrailed* is expressed in the midventral neurogenic region, in the lateral limb budding area, and in the dorsal portion of the forming segments one to six. Stripes seven to nine are restricted to the medioventral part, which includes the neurogenic region (Fig. 1D, E). They consist of seven to nine *engrailed* expressing cells.

Stripes one to seven show a twofold *engrailed* expression in the embryonic epidermis and in the forming ganglia (Figs. 1, 2). In the stages examined, the superficial epidermal *engrailed* expression continues in the forming limbs and the dorsal portions of the segments (Fig. 2). In the neurogenic area of advanced stages, *engrailed* expression is restricted to individual neuronal precursors and neurons, whereas the superficial *engrailed* expression has disappeared (Figs. 1, 2). In contrast to this, stripes eight and nine appear only transiently in the embryonic epidermis (Figs. 1, 2).

Morphogenesis

In all other body regions, the intersegmental furrows are formed immediately posterior to the *engrailed* stripes

(Fig. 1). This is also true for pleonic stripes one to six, but stripes seven to nine are not related to the formation of intersegmental furrows (Figs. 1D, E, 2). In segments one to six, limb buds are formed whose posterior portions are also *engrailed* positive (Figs. 1, 2). No limb buds occur in segments seven to nine, and in the corresponding regions the cells do not show *engrailed* expression (Figs. 1, 2). Interestingly enough, embryonic limb buds are formed in the first pleonic segment (Fig. 1C, D) where the adult *C. destructor*, like all parastacid crayfish species, is devoid of appendages in both sexes (Hobbs, 1988). The limbs associated with the sixth pleonic *engrailed* stripe are clearly the posteriormost appendages on the germ band (Figs. 1C, D, E, 2). On the basis of their shape in advanced developmental stages, they can be identified to be the uropods (Figs. 1, 2).

Neurogenesis

In the pleonic segments one to seven, *engrailed* is expressed in cells of the forming ganglia (Figs. 1, 2). Thereby, the arrangement of *engrailed*-positive cells (neurons?) is very similar between the seventh and more anterior segments (Fig. 2). The *engrailed* expression in stripes eight and nine disappears at about 70% to 75% development and is not correlated with neurogenesis. The staining of the embryonic central nervous system with rhodamine-

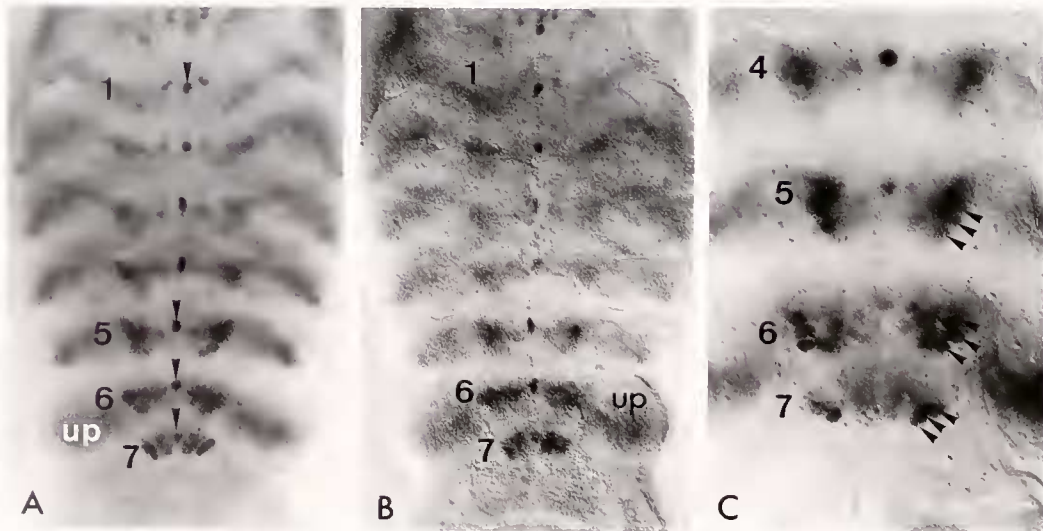


Figure 2. Early neuronal *engrailed* expression in the pleon: up, uropods. (A) Posterior part of the caudal papilla of an embryo at about 75% development (ventral view; brightfield micrograph). The pattern of *engrailed* expression in neuronal precursors and early neurons in the seventh pleonic ganglion anlage is very similar to that of more anterior pleonic ganglion anlagen, indicating a serial homology between all seven pleonic ganglia. The resemblance concerns, in particular, a median *engrailed*-positive cell (arrowheads) and a set of three intensely stained cells at the posterior margin of the *engrailed*-positive area (compare Fig. 2C). Note the dorsal *engrailed* expression in pleomeres one to six. (B) Same preparation as in (A), with Nomarski optics. This technique shows the buds of the pleonic limbs. The uropods are beginning to get their characteristic shape and are clearly connected with the sixth pleomere. (C) Closeup of another preparation (Nomarski optics). The serially homologous group of three intensely stained cells in ganglion anlage seven and more anterior ganglion anlagen is marked by arrowheads.

labeled phalloidin reveals that a true seventh embryonic ganglion is formed in addition to the anterior six pleonic ganglion anlagen (Fig. 3). All pleonic ganglion anlagen one to seven share the occurrence of two main commissures and a characteristic median Y-shaped neuron that might correspond to the neuron S described by Whittington *et al.* (1993) (Fig. 3A, B). Posterior to the seventh, an eighth ganglion anlage is formed. It possesses only one commissure, and the characteristic median cell is lacking (Fig. 3B). Furthermore, no neuronal *engrailed* expression occurs. From this eighth hemiganglion, two nerves run posteriorly towards the embryonic telson region (Fig. 3B). During further ontogenesis the anlagen of ganglia six, seven, and eight fuse and become a morphological unit that forms the terminal ganglion of the adult animals (Fig. 3C). Thereby the commissures remain separated. The coalescence of these ganglion anlagen is clearly visible from about 80% development. However, *engrailed* expression still indicates the composed origin of the terminal ganglion in postembryonic stages (*e.g.*, PO 1) (Fig. 4).

Discussion

Development of the terminal ganglion

The present investigation shows that the terminal ganglion of the adult parastacoid crayfish *Cherax destructor* is the fusion product of three embryonic ganglion anlagen—the sixth and seventh pleonic ganglia and a partial eighth ganglion. This developmental pattern corresponds to that described for the astacoid crayfish *Procambarus clarkii* (Dumont and Wine, 1987). Furthermore, these embryological data are consistent with results from neuroanatomical and immunohistochemical studies analyzing the terminal ganglia of the adults of several freshwater crayfish species (Stoll, 1925; Kondoh and Hisada, 1986; Audehn *et al.*, 1993) and of the lobster *Homarus gammarus* (Winlow and Laverack, 1972). The embryonic morphology and the pattern of *engrailed* expression of the seventh pleonic ganglion resemble to a high degree those of the anterior pleonic ganglia. This suggests that it represents a true segmental ganglion homologous with the anterior segmental ganglia. The eighth pleonic ganglion anlage shows only one commissure. Furthermore, this ganglion lacks any *engrailed* expression which characterizes the posterior part of forming ganglia, although an epidermal eighth pleonic *engrailed* stripe is formed. Taken together, this suggests that the eighth pleonic ganglion of the embryo might be the anterior part of a true segmental ganglion anlage. On the basis of the occurrence of specifically arranged identified neurons, Audehn *et al.* (1993) came to similar conclusions.

The formation of an embryonic anlage of a seventh pleonic ganglion that later fuses with the sixth ganglion has been reported from representatives of most higher

malacostracan taxa including Leptostraca (Claus, 1888; Manton, 1928b, 1934), Hoplocarida (Shiino, 1942), Syn-carida (Hickman, 1937), Mysidacea (Manton, 1928a, b), Tanaidacea (Scholl, 1963), and Isopoda (Strömberg, 1967). No seventh pleonic ganglion occurs in the embryo of the amphipod *Gammarus pulex*; however, for a short time during development, the anlage of the sixth pleonic ganglion is subdivided into two distinct adjacent areas—a phenomenon interpreted as a vestigial formation of a seventh pleonic ganglion (Weygoldt, 1958). None of these authors has mentioned the partial eighth pleonic ganglion, but this might be due to the techniques used (no whole-mounts or horizontal sections). Adult eumalacostracans possess only six pleonic ganglia (see Hanström, 1928), and this is also true for leptostracans with a seventh pleomere (Claus, 1888; Manton, 1928a). Against this background, I conclude that the composite nature of the terminal (sixth) ganglion and the pattern of its formation by fusion during embryogenesis is part of the malacostracan ground plan. Thus, the fusion of the terminal ganglion is apparently not correlated with the evolution of the uropods and their complex function in eumalacostracans.

Origination of the uropods from the sixth pleomere

The anterior six *engrailed* stripes in the embryonic pleon of *Cherax* mark the posterior border of the six pleomeres that persist in the adult. *Engrailed* is expressed in the ventral region comprising the ganglion primordia and the limb buds as well as the lateral and dorsal sides of each segment. The seventh to ninth pleonic *engrailed* stripes are restricted to the ventral side of the embryo. However, from the mode of formation and the distance between them, they correspond to the anterior stripes. Thus I conclude that all pleonic *engrailed* stripes are serial homologues and that stripes seven to nine also indicate segment anlagen.

The pattern of the sixth *engrailed* stripe clearly reveals that the uropods of *Cherax* are the limbs of the sixth pleomere, which is also true for the mysid *Neomysis integer* (unpub. obs.). This result confirms some of the suggestions of Manton (1928a, b), and since there is good evidence that the tail fans of all eumalacostracan groups are homologous (Hessler, 1983; Wägele, 1994), the findings presented here might also be valid for eumalacostracans in general. Therefore, Siewing's (1956, 1963) hypothesis that the uropods originate from the seventh pleon segment cannot be maintained. The present results also contradict the assumption that the caudal rami of the telson of leptostracans are homologous with the eumalacostracan uropods (Bowman, 1971; for further arguments against this view see Schminke, 1976). Furthermore, the telson of decapods does not correspond to the ancestral seventh pleomere, as was suggested by Kondoh and Hisada (1986).

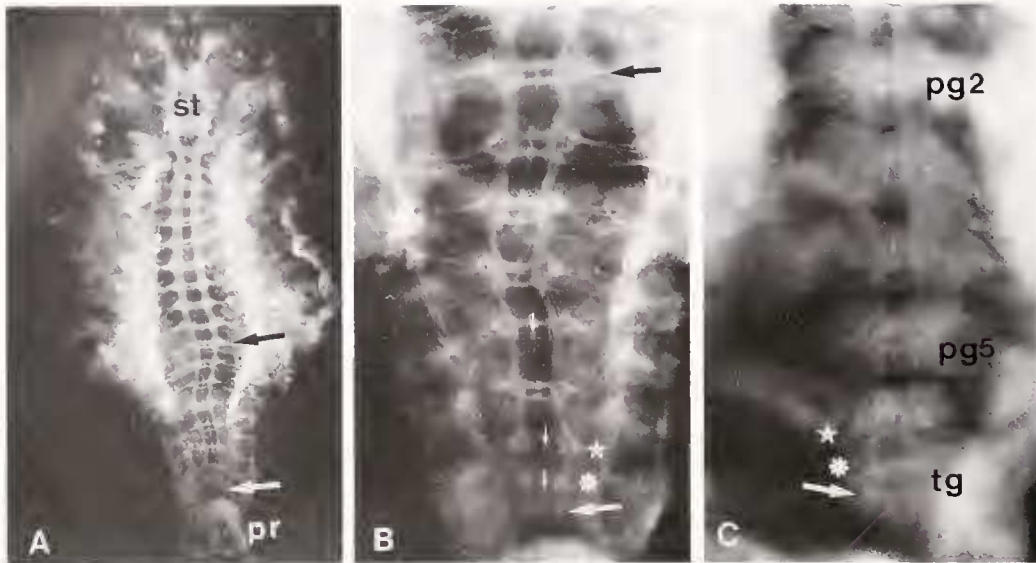


Figure 3. Gangliogenesis as seen with rhodamine-labeled phalloidin: st, stomatodeum; pr, proctodeum; pg2, second pleonic ganglion; pg5, fifth pleonic ganglion; tg, terminal ganglion. (A) The nerve cord of an embryo at 75% to 80% development. The black arrow points to the eighth thoracic ganglion anlage; the white arrow indicates the eighth pleonic (hemi)ganglion anlage. (B) Closeup of posterior ganglion anlagen. The seventh ganglion anlage (asterisk) shows the same pattern as those of more anterior segments (e.g., the sixth ganglion anlage; star). The eighth ganglion anlage (large white arrow) consists of only one commissure. The black arrow shows the eighth thoracic ganglion anlage; the small white arrows point to the median Y-shaped neurons. (C) Advanced stage (85% to 90% development) showing that the sixth (star), seventh (asterisk), and eighth (white arrow) ganglion anlagen are embedded in a morphological unit, forming one large terminal ganglion.

The additional *engrailed* stripes seven to nine lie clearly in front of the telson anlage, which is characterized by the proctodeum and which lacks *engrailed* expression. Against this background, the seemingly "seven-segmented" pleon with uropods originating from the "seventh" pleomere of some lophogastrids is not plesiomorphic, as considered by Manton (1928b), Siewing (1956), and Lauterbach (1975), but is a derived feature. There is ontogenetic and phylogenetic evidence for this suggestion. Manton's (1928b) hypothesis that the uropods migrate posteriorly before the border between the last two segments is formed is not consistent with the finding that the intersegmental furrows are formed before limb buds appear (Scholtz, 1990; present investigation). We know from *Drosophila* genetics that the establishment of the segmental border is the prerequisite for the subsequent differentiation of segments (Lawrence, 1992). Therefore, the "seventh pleonic segment" of lophogastrids is apparently the result of a secondary nonsegmental(?) subdivision of the terminal eumalacostracan segment, as was suggested earlier by Claus (1888). With respect to the position of lophogastrids in the eumalacostracan phylogenetic tree (e.g., Siewing, 1956; Richter, 1993), it is more likely that a subdivision of the terminal segment occurred only once in the lophogastrid line than that the seventh pleomere has been lost independently in several eumalacostracan lines.

The seventh pleonic *engrailed* stripe in the embryo of *Cherax* is an obvious example of the recapitulation of ancestral conditions (see Sudhaus and Rehfeld, 1992). It demarcates the posterior border of an additional (seventh) pleonic segment that is missing in adult crayfish and other eumalacostracans but is present in adult Leptostraca, the sister-group of the Eumalacostraca. There is no reason to assume that pedomorphosis led to the occurrence of the seventh segment in adult leptostracans and to the loss of the highly complex eumalacostracan tail-fan (Hessler, 1983; Paul *et al.*, 1985) in this group.

Interestingly, in the crayfish as in other eumalacostracans, the corresponding seventh pleonic ganglion persists and is fused with the sixth to form a morphological and functional unit (see above). In addition, some authors report that the embryos of several malacostracan species contain terminal mesodermal somites that might be related to a vestigial seventh pleonic segment and that also fuse with the sixth pleonic somites (e.g., Manton, 1928a; Shiino, 1942). Those processes can be characterized as fusions, but fusion does not seem to be the appropriate description for events in the superficial segmental parts. The pleonic *engrailed* stripe seven (like stripes eight and nine) is more like a transient segment anlage that is not involved in morphogenesis

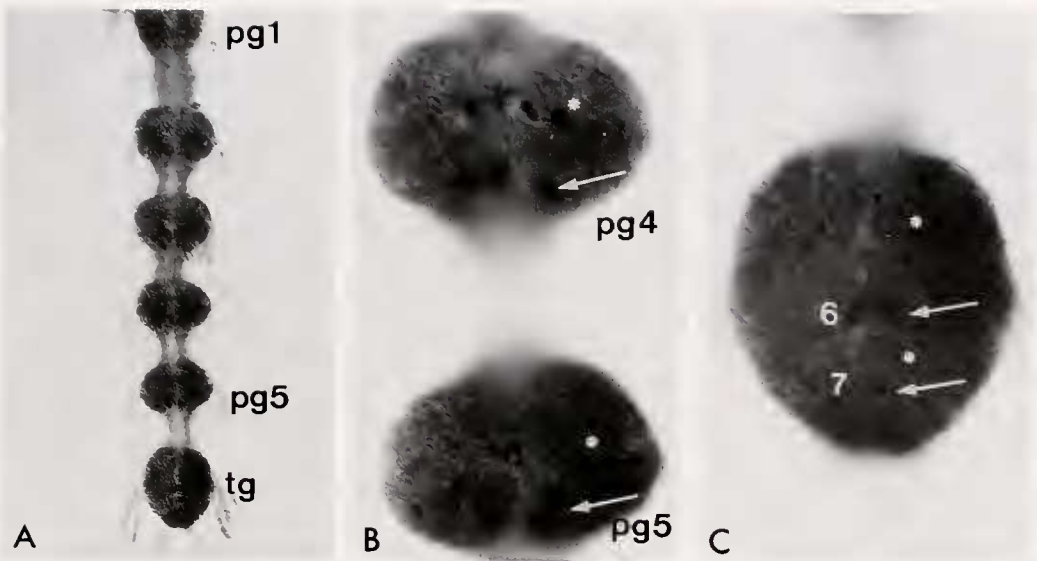


Figure 4. Expression of *engrailed* in the pleonic nerve cord of the first postembryonic stage (PO I): pg1, first pleonic ganglion; pg4, fourth pleonic ganglion; pg5, fifth pleonic ganglion; tg, terminal ganglion. (A) The six ganglia of the pleon of the first stage after hatching (PO I) (ventral aspect). (B) Higher magnification of the same preparation, showing the ganglia of the fourth and fifth pleomeres. They exhibit a serially repeated pattern of two areas of *engrailed* expression. An anterior region of cells (neurons, glia, or their precursors) with relatively small nuclei (asterisks) and a posterior region with large *engrailed*-positive cells (arrows). (C) The terminal ganglion (same preparation as in (A)). The pattern of *engrailed* expression reveals the composed nature of this ganglion. The asterisks indicate the anterior segmental *engrailed* area and the arrows point to the posterior segmental *engrailed* area (compare Fig. 4B). The pattern of *engrailed* expression in the seventh ganglion anlage (7) is somewhat reduced when compared with that of the sixth ganglion anlage (6).

and that disappears during further development. From the outset, the morphological border between the terminal segment and the telson lies behind the sixth pleonic *engrailed* stripe.

Phylogenetic significance of pleonic engrailed stripes eight and nine

The eighth and ninth pleonic *engrailed* stripes are also considered to indicate vestigial segments that recapitulate ancestral conditions. This suggestion is based on the similar appearance of stripes seven to nine and on the fact that many non-malacostracan crustaceans possess more segments than malacostracans. However, it is difficult to say how far back stripes eight and nine point in phylogeny and in which ancestral lineage these segments have been lost in the adults. The question of whether segmentation and tagmatization of the Malacostraca are primitive or are derived within the Crustacea has been debated, and the many attempts to reconstruct the crustacean stem species have yielded very different results concerning tagmosis and segment number. The proposals reach from short animals with only a few segments (Müller, 1864) to forms with many segments (Hessler and Newman, 1975; Lauterbach, 1986), and from forms with a more or less

homonomously segmented trunk (Hessler and Newman, 1975; Schram, 1982; Walossek, 1993) to animals with a distinct subdivision of the trunk into thorax and a limbless abdomen (Lauterbach, 1986; Fryer, 1992). But until the phylogenetic relationships between the higher crustacean taxa are resolved—see Siewing (1963), Schram (1986), and Wilson (1992) for various proposals—the reconstruction of a crustacean ground plan (*sensu* Hennig, 1966) will be pure speculation.

Nevertheless, the present findings permit some tentative conclusions. The occurrence of additional segment remnants in the embryonic pleon of *Cherax* argues against an original number of 15 trunk segments in crustaceans or even mandibulates as suggested by Walossek (1993); the number of trunk segments in the crustacean stem species must have been higher. Therefore, the additional *engrailed* stripes in the pleon of *Cherax* rather argue in favor of Lauterbach's (1975) hypothesis of a loss of posterior segments in the ancestral lineage of malacostracans. The restriction of these additional stripes to the neural region and the entire lack of limb anlagen furthermore support the suggestion that these segments are vestiges of the limbless abdomen postulated by Lauterbach (1986) and Fryer (1992) for the crustacean stem species.

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